REPORT TITLE

Toxicology Response by the Endosulfan Task Force to the Health Effects Division
Risk Assessment for the Endosulfan Reregistration Eligibility Decision Document
Dated February 17, 2000:

Evaluation of Endocrine Disruption Potential for Endosulfan

DATA REQUIREMENT

Not Applicable

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SUBMISSION VOLUME

Volume 3 of 3

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

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Date: January 4, 2001

STATEMENT OF GOOD LABORATORY PRACTICE

The following response is not subject to the principles of 40 CFR 160, Good Laboratory Practice Standards, as promulgated in Federal Register 54, No. 158, 34067-34704, August 17, 1989.

Submitter: _____

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Date: January 4, 2001

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HEALTH EFFECTS DIVISION (HED) RISK ASSESSMENT
FOR THE ENDOSULFAN REREGISTRATION ELIGIBILITY
DECISION DOCUMENT, DATED FEBRUARY 17, 2000

<u>TOXICOLOGY CHAPTER</u>

RE: Endosulfan: HED Risk Assessment for the Endosulfan RED Document (DP Barcode: D250471; Memo by Stephen C. DeVito, Ph.D., dated February 17, 2000) - Exposure Assessment, Section 3.0 "Hazard Characterization" and Related Documents;

Endosulfan079401: Toxicology Chapter for the Reregistration Eligibility Document (HED memo by Nicole C. Paquette, Ph.D. dated November 22, 1999.

The Endosulfan Task Force (ETF), comprised of Aventis CropScience, FMC, and Makhteshim-Agan North America, respectfully submit the following comments in response to the above referenced draft chapter. There are three key areas of concern regarding the EPA's review of the endosulfan toxicity data that the ETF will address. These areas are:

- The NOAEL selection for the 21-day dermal study in rats (Volume 1)
- Requirement of a developmental neurotoxicity study and retention of a FQPA safety factor of 3x due to uncertainty associated with this data gap (Volume 2)
- EPA's suggestion that endosulfan may be an endocrine disruptor (Volume 3)

This volume specifically addresses whether the available literature and guideline studies provide evidence for potential endocrine modulating activities by endosulfan.

I.   INTRODUCTION

In preparation for the final Reregistration Eligibility Decision (RED) on the active ingredient endosulfan, the EPA Health Effects Division (HED) provided the Endosulfan Task Force (ETF) with a draft of their human health risk assessment for all registered uses of this chemical. Supporting documents for this risk assessment included the Hazard Identification Assessment Review Committee (HIARC) Toxicology Chapter, the HIARC report on toxicological endpoints for risk assessment, the FQPA Safety Factor Committee report, and literature review by Dr. David Liem on the potential of endosulfan to be an endocrine disruptor. On May 10, 2000, the ETF submitted an initial 30-day response identifying errors in the draft risk assessment and providing brief summaries on issues of concern regarding the selection of toxicological endpoints, application of FQPA safety factors and implications by the Agency that endosulfan has the potential to be an endocrine disruptor.

The purpose of this submission is to further elucidate the areas of concern discussed briefly in the 30-day response. This volume specifically addresses the Agency's review of endosulfan data

and the subsequent evaluation of endosulfan's potential as an endocrine disruptor (Liem 98). As part of this response, the ETF has conducted a thorough review of available guideline data and public literature with regard to the potential endocrine modulation activity of endosulfan. The remainder of this document provides a summary of this review and a weight-of-evidence evaluation of the potential of endosulfan to cause endocrine modulation in intact organisms. The ETF based this evaluation on the current working definitions of an endocrine disruptor as defined by national and international scientific bodies such as the Endocrine Disruption Screening and Testing Advisory Committee (EDSTAC) and the Organisation for Economic Co-operation and Development (OECD) Task Force for Endocrine Disruptor Testing and Assessment (EDTA). As discussed in the following sections, an appropriate evaluation of a chemical must be based on an assessment of all available screening (*in vitro* and *in vivo*) and testing (*in vivo*) data, with significant weight given to information derived from testing in intact organisms. This is in accordance with current scientific consensus as reflected in the following working definitions for endocrine disruptors.

II.   DEFINITION OF AN ENDOCRINE DISRUPTOR

In order to address concerns regarding endocrine disruption in the environment, regulatory agencies around the world have initiated both national and international scientific investigations into the development and validation of screening and testing methods to evaluate chemicals for endocrine disruption. In 1996, EPA established the EDSTAC to assess and validate available methods for the purpose of determining whether a chemical should be regulated as an endocrine disruptor. The European Union (EU) under the OECD also established a scientific Task Force to address endocrine disruption, EDTA. The first step in this process for each of these committees was to establish a working definition of an endocrine disruptor.

A. OECD Definition

OECD and EU agreed at the Weybridge workshop (1996) on the following definition:

"An endocrine disrupter is a exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function". (OECD 1997)

B. EPA/EDSTAC Definition

EDSTAC agreed the following description:

"An endocrine disruptor is an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or sub-populations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle." (EDSTAC 1998)

Following these definitions, the committees agreed that the most appropriate method for evaluation of chemicals was through a tiered approach, starting with validated screening assays

and progressing to full *in vivo* studies. The committees also emphasized that definitive determination of endocrine disruption potential must be made through evaluation of data derived in intact organisms.

There is general scientific agreement that the *potential* of a chemical to cause endocrine disruption may be initially assessed using *in vitro* and *ex/in vivo* screening models. However, there are limitations to the predicitiveness of these types of assays, particularly the *in vitro* assays, which are incapable of replicating the intricacies of a biological system. *In vivo* screening models are more predictive, but also tend to focus on one or a few aspects, e.g. the uterotrophic assay mainly on uterus weight as a general measure for estrogenic activity. *In vivo* studies, where a functional endocrine system is present and the full interplay between normal physiological and biochemical processes occurs, provide the most definitive assessment of a chemical's potential for endocrine modulation. Studies that specifically evaluate sexual maturation, fertility and other reproductive endpoints, endocrine organ effects and generation-to-generation effects provide the most significant scientific evidence for regulatory purposes.

As stated previously, the potential of endosulfan to cause endocrine modulation has been evaluated based on these international definitions of an endocrine disruptor, and the weight-of-evidence determination was based on the concepts presented above. The data supporting this assessment is presented in the following section.

III. EVALUATION OF ENDOSULFAN DATA

In both the draft HED chapter and the appended review by Dr. Liem, the Agency stated that experimental evidence exist which generated concern regarding the potential of endosulfan to cause endocrine modulation. This concern was centered on potential hormonal interactions and endocrine organ effects noted in public literature and mammalian toxicity studies. Most of the data presented by the Agency was based on *in vitro* and *in vivo* screening assays. As stated previously, the *in vitro* test systems give some indication of the potential binding to human-like estrogen, progesterone or androgen receptors and of the response of these receptors, however, they have limited predictive value for the real situation in living organisms, which have a complex endocrine regulation.

Extensive research by regulatory agencies, industry and academia has been conducted to determine the most reliable and predictive methods for evaluation of chemicals for endocrine disruption potential. Many of the initial *in vitro* screening assays used to assess chemicals have been found to be too unreliable for predicting endocrine activity of chemicals in biological systems and will not be used for future regulatory screening of chemicals (e.g. MCF-7 cell proliferation assay). Current efforts by OECD, EPA and other global regulatory agencies are being made to assess and validate *in vitro* and *ex/in vivo* methods such as the uterotrophic and Hershberger assays for regulatory screening purposes.   Even with the ongoing development of validated screening assays, *in vitro* and *ex/in vivo* tests are not suited in isolation for hazard evaluation and risk assessment, since they focus on one test parameter only.

In contrast, most regulatory agencies agree that the extensive data package of *in vivo* toxicity studies on regulated plant protection products provides the most appropriate information for a

scientifically based hazard and risk evaluation of endocrine effects on reproduction and development in humans. Therefore, a scientifically sound evaluation of a chemical's potential to cause endocrine modulation must be based on a weight-of-evidence evaluation of all available *in vitro and in vivo* screening and *in vivo* studies, with the most weight assigned to valid, guideline *in vivo* studies. As stated previously, this conclusion is supported by the currently accepted OECD and EPA definitions for endocrine disrupters.

The following sections summarize the available public literature and guideline studies for endosulfan. While the *in vitro* data show a very weak binding potential of endosulfan to estrogen and progesterone receptors, subsequent data from four different *in vivo* uterotrophic assays were negative. These data are significant since EPA and OECD are currently working to validate the uterotrophic assay for regulatory screening purposes. In addition, an overall evaluation of the *in vivo* toxicity studies performed to GLP guidelines shows no indication of endocrine-related adverse effects.

A. Endocrine Modulation: *in vitro* screening assays

EPA's literature review (Liem 98) evaluated two areas of endocrine activity: 1) hormonal changes, as assessed by *in vitro* and *ex vivo* screening assays; and 2) effects in endocrine organs. With respect to hormonal changes, the review cited several *in vitro* assays demonstrating potential estrogenic, androgenic and progesteronic effects by endosulfan. The following table contains a more inclusive summary of the available literature describing *in vitro* screening of endosulfan estrogenic and progesteronic binding potential:

Table 1. Endosulfan: *In Vitro* Studies on Endocrine Effects

Type of *in vitro* Study	Endpoint	Endocrine Effects
MCF-7 Cell proliferation assay (Soto et al. 1995)	Cell proliferating potency	10^6 times less effect than 17β-estradiol
MCF-7 cell proliferation assay (Wade et al. 1997)	Cell proliferating potency	Effect **Ca.** 10^6 less than l7β-estradiol Only effect at highest soluble dose $5x10^{-5}$M.
MCF-7 Cell proliferation assay (Arcaro et al. 1998)	Cell proliferation	Effect Ca. 10^6 less than 17β-estradiol. Only at highest dose ($l0^{-5}$ M) an effect.
MCF-7 to progesterone receptor binding assay (Soto et al. 1995)	Relative binding affinity to hPR	Binding Ca. 10^5 less than 17β-estradiol
MCF-7 binding assay to human estrogen receptor (hER) (Soto et al. 1995)	Relative binding affinity to hER.	Binding $2.4x10^6$ less than 17β-estradiol
Transcriptional activation in HeLa-cells transfected with mouse ER and ERET81CAT (Shelby et al. 1996)	Relative binding affinity to mER	No binding at highest dose (10^{-6} M)
Yeast expression of hER (Ashby 1997)	Relative binding affinity to hER	At 10^{-5} M no binding
Yeast BJ2407 expression of human ER (Ramamoorthy et al. 1997; Gaido et al. 1997)	Relative binding affinity to hER	At 10^{-5} M no increase; At 10^{-4} M similar binding as 10^{-9} M DES, i.e. l0^5 times less binding than DES
Yeast BJ2168 expression of mouse ER (Ramamoorthy et al. 1997)	Relative binding affinity to mER	l0^5 times less binding than DES
Endosulfan: Evaluation of Possible Endocrine Effects in Fish: Lab Project (Heusel, R. 1999)	Vitellogenin gene expression	Endosulfan was negative for vitellogenin induction, even at levels that were toxic to the target organism.

Based on the results shown in Table 1, endosulfan has been shown to cause extremely small effects on cell proliferation and very limited binding efficiency to estrogen and progesterone receptors *in vitro*. Endosulfan achieved similar effects as the natural hormone estrogen only when the concentrations were 10^5 to 10^6 times higher, indicating a very weak estrogenic potential *in vitro*.

B. Estrogenic *In Vitro* and *Ex Vivo* Screening Assays

The uterotrophic assay and the receptor binding studies published by Wade et al (1997) show that the slight estrogenic effects seen *in vitro* did not occur *in vivo,* even at sublethal doses. Three more uterotrophic assays have been published, each indicating a lack of endocrine effect at maximum tolerated doses. As stated previously, the results of these studies are significant since EPA and OECD are in the process of completing the validation of this assay for future regulatory screening purposes.

Table 2. Endosulfan: *In vivo* and *ex vivo* Estrogenic Assays

Type of *in vivo* study	Endpoints	Endocrine Effects
Competitive binding to rat uterus ER *ex vivo* (Wade et al. 1997)	estradiol binding to rER '	Endosulfan inhibits estradiol binding only at excess. The number of ER and PR in uterus was unchanged
Competitive binding to mouse uterus *ex vivo* (Shelby et al. 1996)	estradiol binding to mER	No competitive inhibition at 10^3 fold excess
Uterotrophic assay in sexually immature Sprague-Dawley rats (3 mg/kg/day i.p. on day 18-20 of age) (Wade et al. 1997)	Uterus: growth, peroxidase activity, number of PR/ER; Pituitary: weight, hormones (GH, prolactin, TSH, LH, FSH); Serum: Thyroxin	No uterotrophic activity or hormonal changes. DES caused increase in uterus weight (80%), peroxidase, prolactin and a decrease in number of ER
Uterotrophic assay in sexually immature CD 1-mouse (10 mg/ kg bw/day s.c. on days 17 -19 of age) (Shelby et al. 1996)	Uterine growth	No increase in uterine wet mass. DES, E_2, (4-OH)-tamoxifen, DDT, methoxychlor were positive
Uterotrophic assay in sexually immature AP-Wistar rats (5 - 100 mg/kg bw/day s.c. for 3 days) (Ashby et al. 1997)	Uterine growth	No increase in uterine wet mass. Estradiol and methoxychlor were clearly positive.
Uterotrophic assay on young ovariectomized female Wistar rats (Raizada et al. 1991)	Uterus / cervix / vagina wet weight and glycogen content; pituitary weight; histology	No effects after gavage of 1.5 mg/kg bw/day for 30 days although transient clinical signs were present.

The binding potency of endosulfan to estrogen receptors in homogenized uterus tissue *ex vivo* was 5-6 orders of magnitude lower than that of the natural hormone, supporting the evidence of negligible binding potential from the *in vitro* assays.

C. Androgenic *In Vitro* and *Ex Vivo* Screening Assays

The agency (Liem 98) also cited several screening assays and *in vivo* studies investigating potential anti-androgenic effects of endosulfan. A summary of this data is presented in the following table.

Table 3. Endosulfan: *In vivo* and *ex vivo* Androgenic Assays

Study	Endpoints	Results
Crl-CDI mouse dietary dose at 0, 3.8, 7.5, 15 mg/kg bw for 7 days (Wilson et al. 1997)	Liver toxicity; steroid hormone metabolism	At 7.5 mg/kg: Males had bw loss and stress; At 3.8 mg/kg: Females: steroid metabolism ↑, urinary androgen clearance ↑; Serum hormones unchanged at all doses
Adult male Wistar rat gavaged 0, 2.5, 5.0, 7.5 or 10 mg/kg for 7 or 15 (Singh and Pandey 1989a)	Testis: GST, Testosterone Serum: Testosterone	A "variable" effect on testosterone production is claimed. This effect was not dose related
Adult male Wistar rats gavaged 7.5 or 15 mg/kg for 15 and 30 days (Singh and Pandey 1989b)	Liver enzymes involved in testosterone metabolism; Serum/Liver testosterone	Cytochrome P450 induction, steroid metabolism ↓, Changes only after 30 days and reversible. No change in liver/body wt.
Adult male Wistar rats gavaged 7.5 or 15 mg/kg for 15 and 30 days (Singh and Pandey 1990)	Liver enzymes involved in testosterone metabolism; Serum: testosterone, FSH, LH; Testis: testosterone	CytochromeP450 induction, steroid metabolism, Serum/testis testosterone/FSH, LH ↓; Changes all reversible. No change in liver/testis/body wt.
Adult male Swiss mice 0, 9.8, 12.7 or 16.6 mg/kg i.p. for 5 days (Pandey et al. 1990)	Dominant lethality weekly at 1 to 8 weeks after dosing	Dominate lethality only at week 6 only at the high dose. No effect in any other week
Acrosome Reaction (AR) in capacitated human sperm *ex vivo* (Turner et al. 1997)	Staining of the inner acrosomal membrane; sperm mortality,	Inhibition of AR by pre-treatment with 1 nmol. No effect on AR at 1 nmol in ovarian follicles *in vivo*. Sperm motility not affected.
Adult male Druckrey rats gavaged 0, 2.5, 5.0, 10.0 for 70 days (Sinha et al. 1995)	Testis: SDH, LDH, GGT, G6PDH, sperm/spermatid count and morphology	Sperm (atid) count ↓, SDH ↑, GGT ↑, G6PDH ↑, LDH ↑. Increased incidence of sperm abnormal morphology from 6.3%(control) to 7.2% was stated significant
Weanling male Druckrey rats 0, *2.5*, 5.0, 10.0 for 70 days (Sinha et al. 1997)	Testis: SDH, LDH, GGT, G6PDH, sperm and spermatid count and morphology	Sperm (atid) count ↓; SDH ↓, GGT ↑, G6PDH ↑, LDH ↑; Increased incidence of sperm abnormal morphology from 6.3% (control) to 8.1% was stated as significant

The screening studies for androgenic effects are inconclusive. In most cases details on the methods and characterization of the test substance were often not adequately defined. Moreover, isolated findings such as testicular atrophy, characterized by degeneration and necrosis of seminiferous tubules, increased steroid metabolism and reduced sperm count have been claimed, but these results are not in line with the outcome of valid reproduction studies. Reproductive and developmental studies in a number of species did not reveal any effect on reproduction indices (such as fertility), nor any increase in the incidence of defects or abnormalities in offspring (Tables 4 & 5). Most of the effects noted in the studies in Table 3 are mostly likely related to the frank toxicity of endosulfan, and therefore, the functional significance of these findings is unclear and of limited significance to humans.

In cases where the doses were high enough to produce serious intoxication, the observed endocrine effects were likely secondary to adverse effects at a non-endocrine target tissue. One example of this involves toxicity to the liver, which then has a distal effect on the endocrine system. The effects of endosulfan on the liver are well documented, where exposure to high dose levels markedly induces microsomal enzyme activity. Induction of enzyme activity can increase metabolic clearance of endogenous hormones, resulting in

lower blood levels and subsequently a compensatory increase in pituitary hormone secretion to maintain homeostasis within an endocrine axis. Enzyme inducers are also known to have effects on the hepatic metabolism and clearance of steroids such as corticosterone. They can also affect androgen-metabolizing enzymes and as such may indirectly affect a number of other major endocrine axes, such as the pituitary - adrenocortical and pituitary - gonadal axes. The mechanism by which a range of liver microsomal enzyme inducers cause thyroid function changes and pathology, including carcinogenesis, is now well understood to be an entirely indirect mechanism that has little relevance to humans.

Therefore, as a result of toxicity elsewhere in the organism, secondary endocrine effects may be functionally and mechanistically linked to alteration in physiological homeostasis. The literature studies with positive findings clearly failed to verify the causal-effect relationship to changes in endocrine function.

On the other hand, a large number of proprietary *in vivo* mammalian toxicity studies and published uterotrophic assays indicate that Endosulfan does not elicit modulation of any endocrine organ or system (Tables 2, 4 and 5). Neither morphological nor functional effects on endocrine or reproductive organs, nor any effect on reproductive performance, sexual development, differentiation or maturation, nor activity related to any other endocrinological endpoints was found, even though doses in these studies were applied in the toxic range (Tables 4 & 5)

D. Endocrine Modulation: Information from *in vivo* studies

In the registration process of endosulfan, hazard evaluation and risk assessment was performed on a wide range of effects, including reproductive effects. This set of toxicity and metabolism studies of endosulfan has been reviewed by regulatory agencies in registration processes worldwide. In addition, all data of endosulfan have been evaluated international bodies such as JMPR (WHO/FAO). The toxicity studies contain all the information necessary to evaluate the potential of endocrine effects. The endpoints relevant to endocrine modulation in these tests have been listed and explained by Stevens et al. (1998) based on the criteria established by ECPA (1996). For endosulfan the complete array of *in vivo* toxicity studies is available. These studies were carried out using a wide spectrum of doses including the maximum tolerated dose (MTD). The parameters relevant for endocrine effects in adults and offspring, measured in these studies, are summarized in Tables 4 and 5.

Table 4: Endosulfan - Endocrine endpoints in required toxicity studies *in vivo:* Adults

Endpoints	Subchronic .				evelopmental		2.Gen. Repro.	Chronic/ Carcinogenicity	
OECD-Guideline Number	408		410	452	414		416	453	
Species	Rat	Mouse	Rat	Dog	Rat	Rabbit	Rat	Rat	Mouse
Reproduction							Neg.		
Fertility							Neg.		
Fecundity							Neg.		
Gestation length					neg.	neg.	Neg.		
Abortion					neg.	neg.	Neg.		
Premature Delivery					neg.	neg.	Neg.		
Difficult labor							Neg.		
Time to mating.				.			Neg.		
Mating and sexual behavior							Neg.		
Estrus cycle							Neg.		
Ovulation	neg.	neg.	neg.	neg.			Neg.	neg.	neg.
Spermatogenesis	neg.	neg.	neg.	neg.			Neg.	neg.	Neg.
Sperm count									
Gonad development	neg.	neg.	neg.	neg.	neg.	Neg.	Neg.	neg.	Neg.
Secondary sexual characteristics (muscle mass)	neg.	neg.	neg.	neg.			Neg.	neg.	Neg.
Gross pathol. of repro. Organs	neg.	neg.	neg.	neg.	neg.	neg.	Neg.	neg.	Neg.
Histology reproductive organs	neg.	neg.	neg.	neg.			Neg.	neg.	Neg.
Hormone levels									
Major sex differences	neg.	neg.	neg.	neg.			Neg.	neg.	neg.
Endocrine tumor incidence	neg.	neg.	neg.	neg.			Neg.	neg.	neg.

There were effects cited by Liem from a 1978 National Cancer Institute (NCI) in rats that showed testicular atrophy and parathyroid hyperplasia. However, these results were most likely due to frank systemic toxicity that was seen at both the low and high dose. Male rats in both dose groups showed significant renal and liver toxicity, as well as mortality rates of 38% and 50%. As stated previously, severe intoxication which involves organs such as the liver and kidney results in significant disruption of physiological homeostasis and indirect effects on the major endocrine axes. In addition, there is no indication of these types of effects occurring in guideline accepted chronic studies in rats where the MTD was met, but not exceeded.

Table 5. Endosulfan - Endocrine endpoints in required toxicity studies *in vivo:* Offspring

Endpoints	Developmental Toxicity		2-Generation Reproduction
OECD-Guideline Number	414		416
Species	Rat	Rabbit	Rat
Sexual differentiation	Neg.	Neg.	neg.
Offspring sex ratio	Neg.	Neg.	neg.
Gonad development (size, morphology, weight)	Neg.	Neg.	neg.
Accessory sex organ development	Neg.	Neg.	neg.
Accessory sex organ function (secretory chems.)	-	-	neg.
			neg.
Sexual development/maturation (vaginal			neg.

Endpoints	Developmental Toxicity		2-Generation Reproduction
OECD-Guideline Number	414		416
Species	Rat	Rabbit	Rat
opening, testes descent (cryptorchidism), preputial separation, nipple development)	neg.	neg.	neg.
Malformations genital tract	neg.	neg.	neg.
Gross pathology of reproductive tissues	-	-	neg.
Histology reproductive tissues .	-	-	neg.
Viability of the conceptus			neg.
Viability of the offspring (neonatally)	neg.	neg.	neg.
Growth of the conceptus (weight)	neg.	neg.	neg.
Growth of offspring	-	-	neg.
Major sex differences	neg.	neg.	neg.

The *in vivo* toxicity studies unequivocally show that endosulfan does not cause endocrine activity:

a) Subchronic studies on rats, mice and dogs: Hormone levels were not measured in these studies. However, the major consequences of hormonal changes were determined: organ weight changes of the endocrine organs such as pituitary, uterus, ovaries, adrenals, mammary gland, testes, thyroid, epididymides, seminal vesicles, vagina. No effects were found on endocrine or reproductive organs (MRID 00145668, 00147182, and 41099501).

b) Chronic studies on rats and mice: In lifetime exposure studies, minor hormone related effects of a test substance would become evident. However, in guideline acceptable studies endosulfan did not cause any changes or increased tumor incidence in endocrine or related organs (MRID 41099502 and 40792401)

c) Developmental toxicity studies on rats and rabbits: Treatment during organogenesis did not affect the development and maturation of any endocrine system (MRID 43129101 and 00094837).

d) Two generation reproduction study on rats: This study measures possible disturbances of reproductive performance, development and maturation including development of sex organs (vaginal opening, testis descent, cryptorchidism, etc.) at doses up to and including parental toxicity. Endosulfan, administered to both male and female rats, did not cause such interference through two successive generations (MRID 00148264). There was an indication of weight effects on the pituitary gland of the F_0 pups of the first mating and uterus of the F_{1b} pups from the first mating. These effects are of limited significance since neither the pituitary or uterus was seen as a target organ in any other study, there was no supporting histopathological changes noted, nor were these effects consistent across generations. In addition, four separate uterotrophic assays were negative for uterine effects at doses up to 100 mg/kg bw/day, suggesting that the weight-of-evidence is negative for specific endocrine effects on the uterus. Lastly, the statistically significant increase in pituitary weights was due to a single female in the high dose group. Therefore, the results indicate that endosulfan does not cause disruption of the endocrine system in parents or offspring at dietary dose levels up to and including 75 ppm (3 - 6 mg/kg bw/day), a toxic level in adult animals. Based on the results summarized above, the evidence clearly shows that endosulfan is negative for all endocrine-related effects.

E. Endocrine Effects of Mixtures of Pesticides

Lastly, there has been public and regulatory concern regarding the endocrine hazard potential of chemical mixtures. Arnold et al. (1996) reported dramatic synergism by a factor 100- to 1 600-fold with weakly estrogenic chemicals tested together in the *in vitro* genetically engineered yeast cell culture system. One year later the report was retracted (McLachlan 1997), because the authors themselves as well as many other laboratories (Table 6) could not reproduce the results. The data summarized below in Table 6, clearly demonstrates that endosulfan does not interact with or alter the potency of known or suspected endocrine disruptors.

Table 6: Endosulfan - Synergy in endocrine effects with other chemicals

Study	Combination chemicals	Synergistic effect
In vivo tests		
Uterotrophic assay in sexually immature AP-Wistar rats (Ashby et al. 1997)	Dieldrin	No effect with either chemical or combination
Competitive binding to rat uterus estrogen and progesterone receptor *ex vivo* (Wade et al. 1997)	Dieldrin	Additive
In vitro tests		
MCF-7 Cell proliferation assay (Soto et a!. 1994/1995)	DDD, dieldrin, tetrachlorobiphenyl, hexachlorobiphenyl, p.p'-DDT, p,p'- p,p'-DDE, methoxychlor, toxaphene,	Additive
MCF-7 cell proliferation assay (Wade et a!. 1997)	Dieldrin	Additive
MCF.7 cell proliferation and hER binding assay (Arcaro et al. 1998)	Dieldrin	Additive
Yeast expression of hER (Ashby 1997)	Dieldrin	No activity at all
Yeast BJ2407 expression of human ER (Ramamoorthy et al. 1997; Gaido et al. 1997)	Dieldrin, Toxaphene, Chlordane	Antagonized by Chlordane/Toxaphene Additive with Dieldrin
Yeast BJ2 168 expression of mouse ER (Ramamoorthy et al. 1997)	Dieldrin, Toxaphene, Chlordane	Antagonized by Chlordane/Toxaphene Additive with Dieldrin

The studies on synergism unequivocally show, that underestimation of the estrogenic potency of a single chemical, due to synergistic interaction with other agents, is very unlikely. The tests also clearly indicate absence of synergy of endosulfan with various organochlorine insecticides.

IV. CONCLUSION

The ETF believes that the data for endosulfan is complete and reliable, including four uterotrophic assays, which is the same assay currently undergoing validation for use as a regulatory screen. The weight-of-evidence from *in vitro* and *in vivo* screening tests and *in vivo* toxicity tests clearly show that endosulfan is not an endocrine disruptor. The ETF believes that until EPA established their own set of criteria for determining endocrine-related effects and has the opportunity to fully evaluate the available data for endosulfan, allegations concerning its potential as an endocrine disruptor should be deleted from the RED.

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